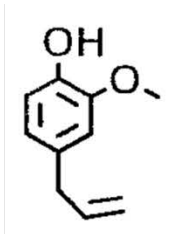
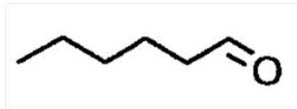


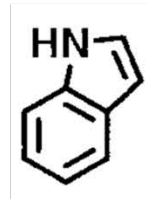
# Os mecanismos moleculares do olfato: a deleção do gene *Ric-8b* resulta em um camundongo anósmico



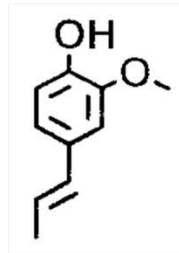
clove



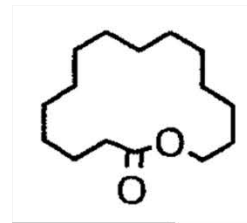
grass



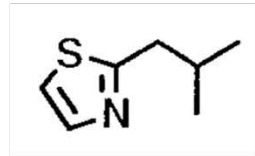
cadaver



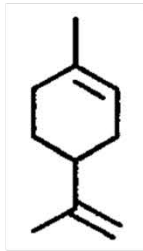
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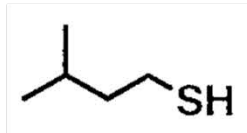
musk



tomato



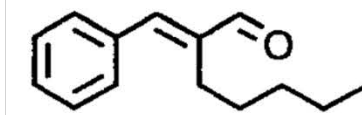
lemon



skunk



camphor



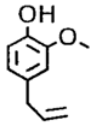
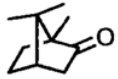
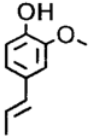
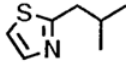
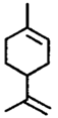
jasmin

Bettina Malnic

Departamento de Bioquímica  
Universidade de São Paulo

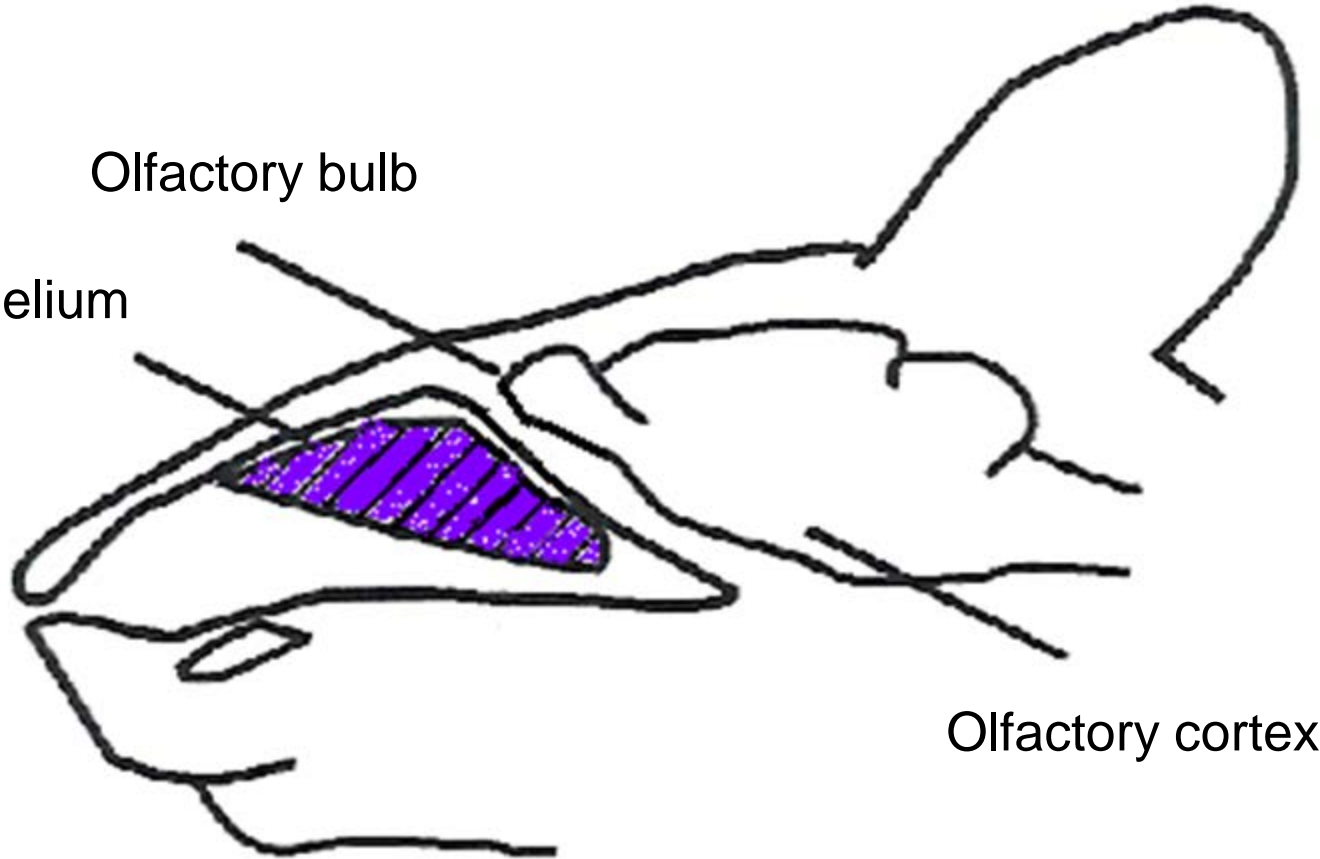


# Olfactory system



Olfactory bulb

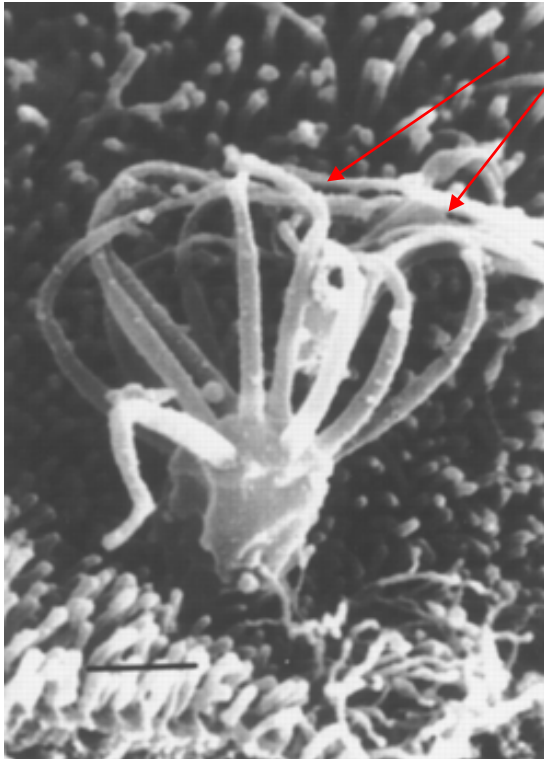
Olfactory epithelium



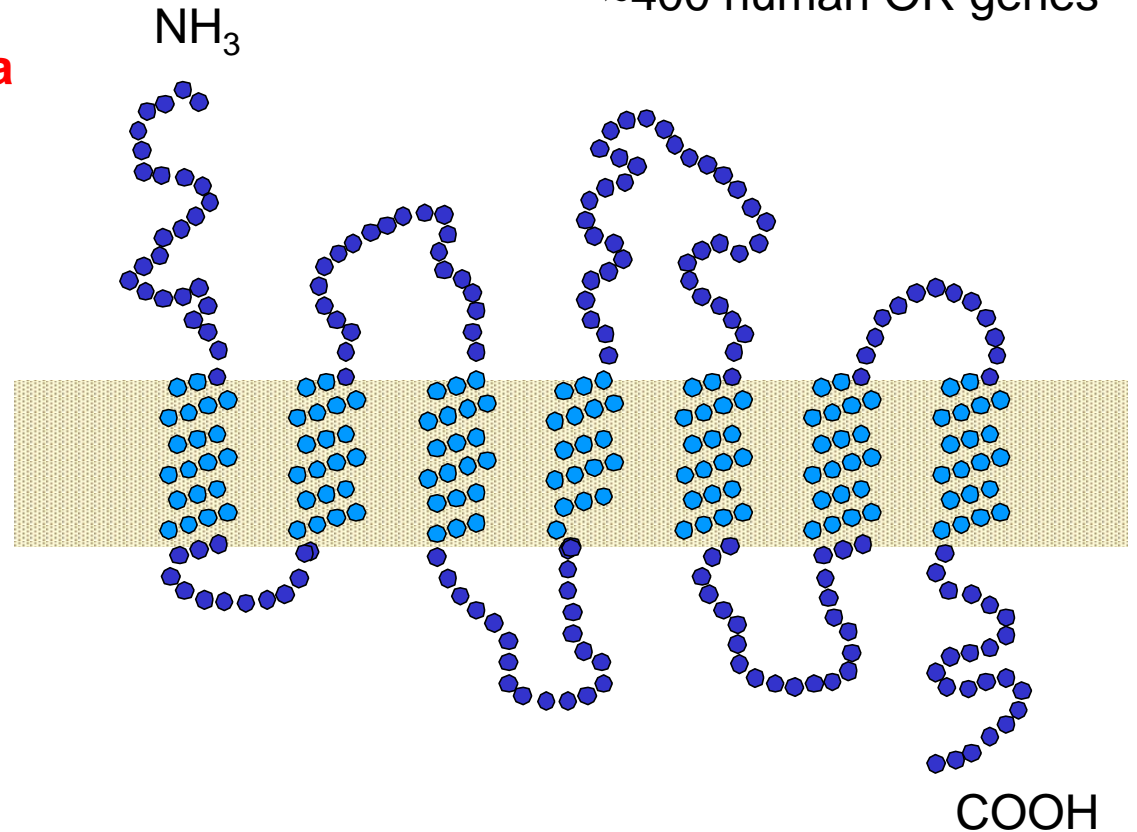
Olfactory cortex

# Odorant receptors are expressed in the cilia of olfactory sensory neurons

~1000 mouse OR genes  
~400 human OR genes

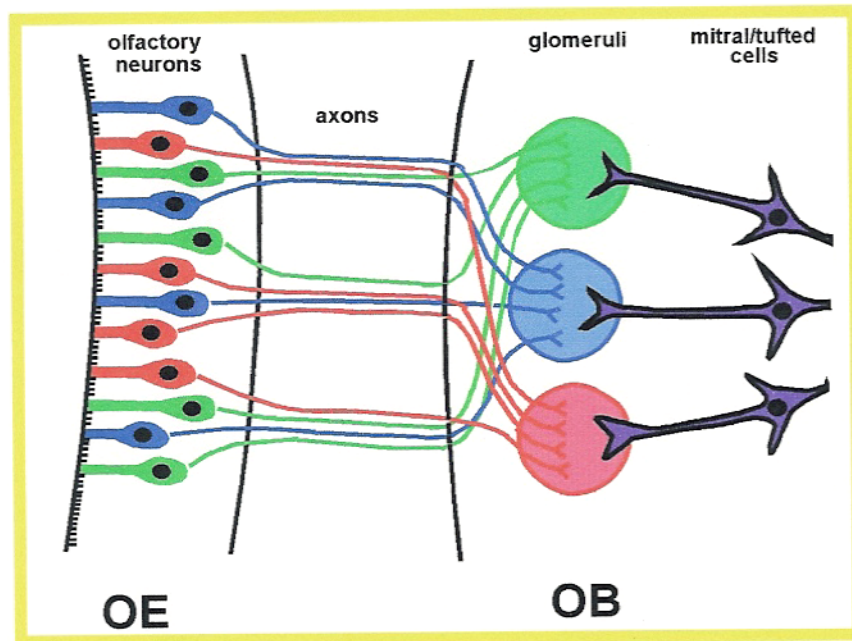
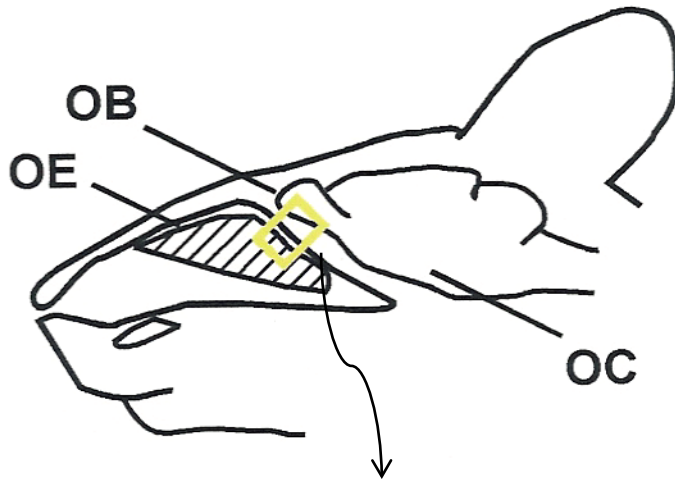


cilia



olfactory sensory neuron

# A map for odorants in the brain





## The Nobel Prize in Physiology or Medicine 2004

"for their discoveries of odorant receptors and the organization of the olfactory system"

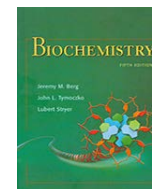
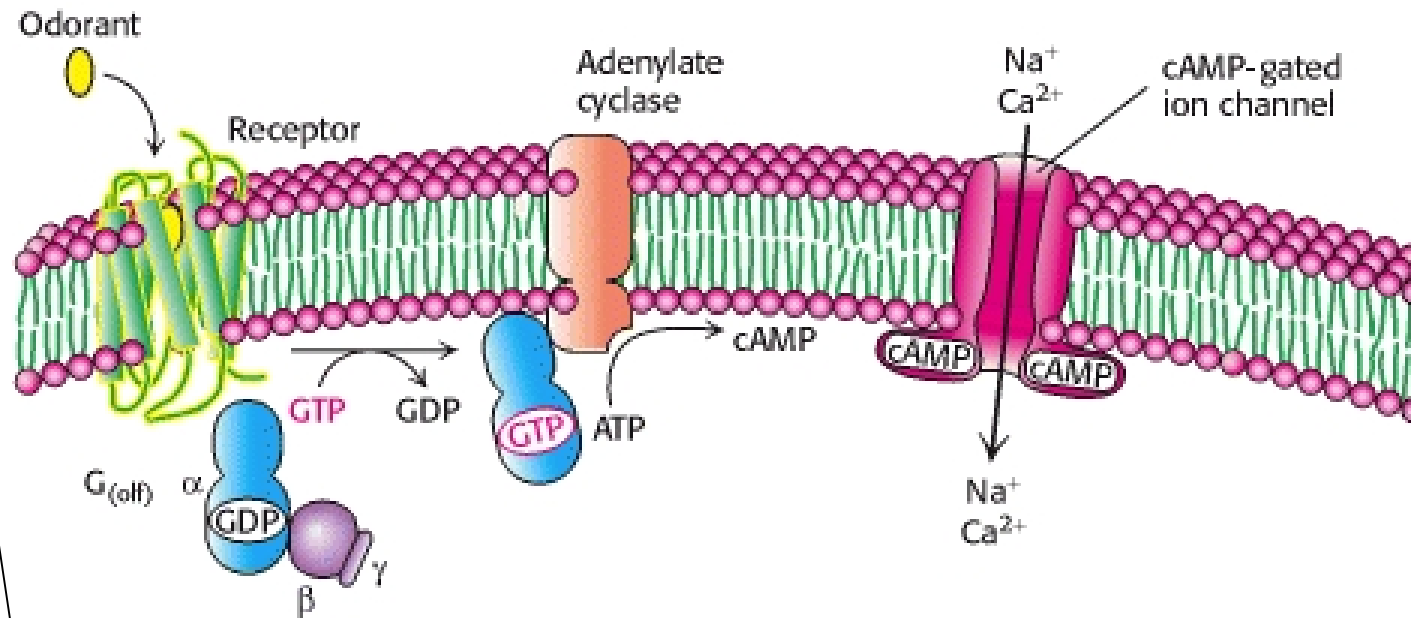
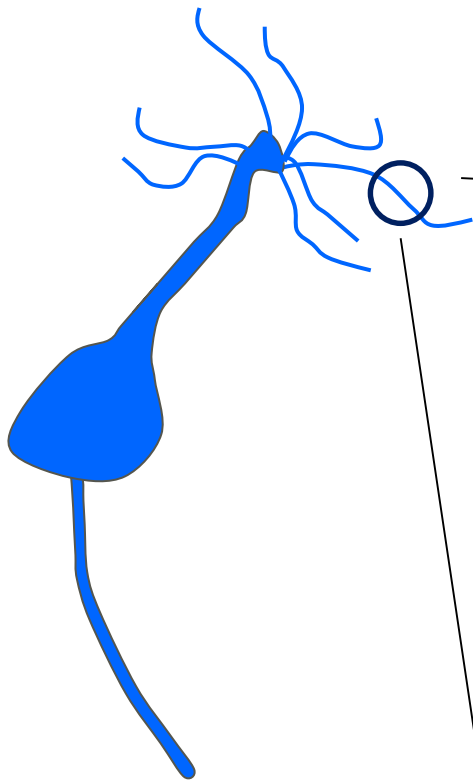


**Richard Axel**



**Linda B. Buck**

# The odorant signaling pathway

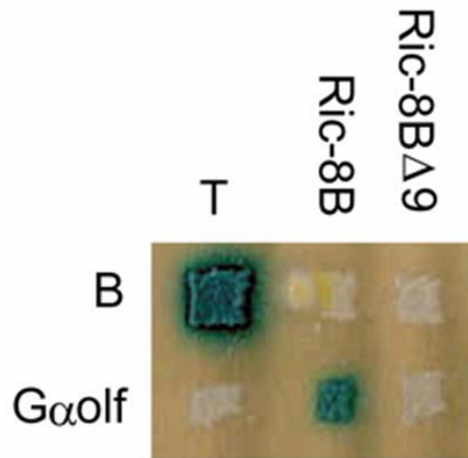


Biochemistry. 5th edition.  
Berg JM, Tymoczko JL, Stryer L.  
New York: WH Freeman; 2002.



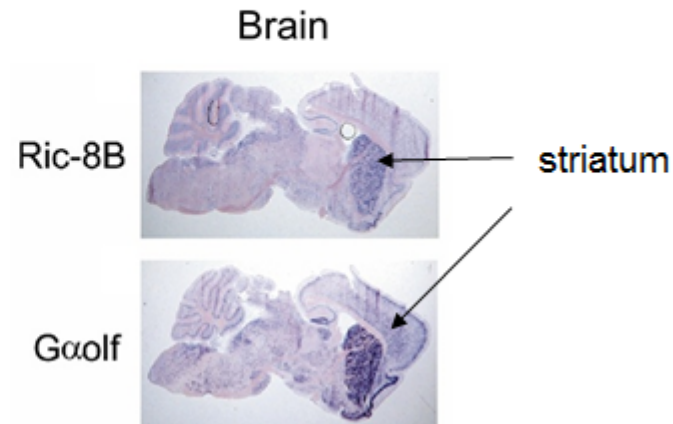
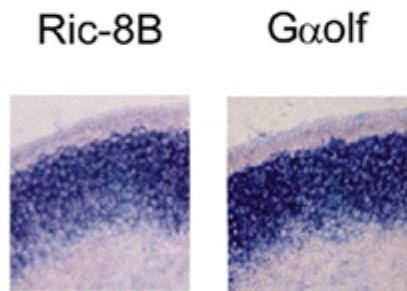
# Ric-8B interacts with G $\alpha$ olf

Y2H screening of an OE cDNA library

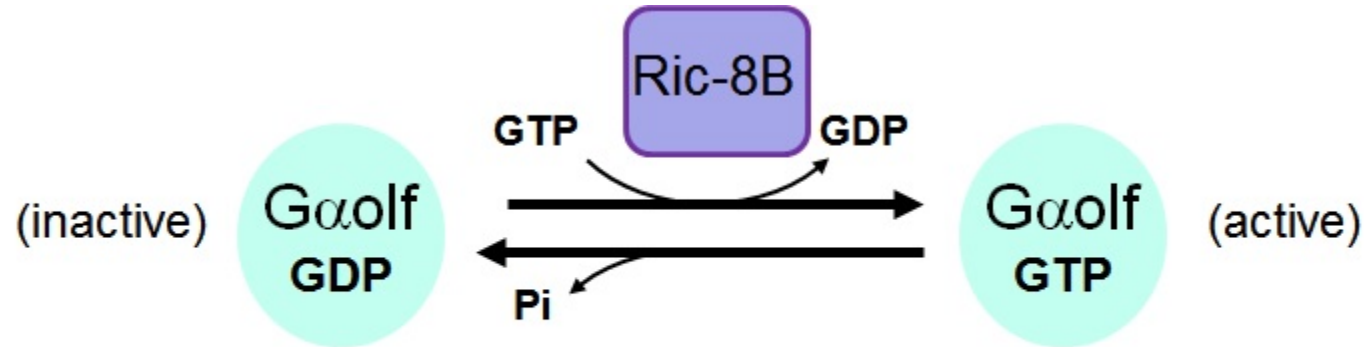


## Ric-8B is coexpressed with G $\alpha$ olf

Olfactory epithelium

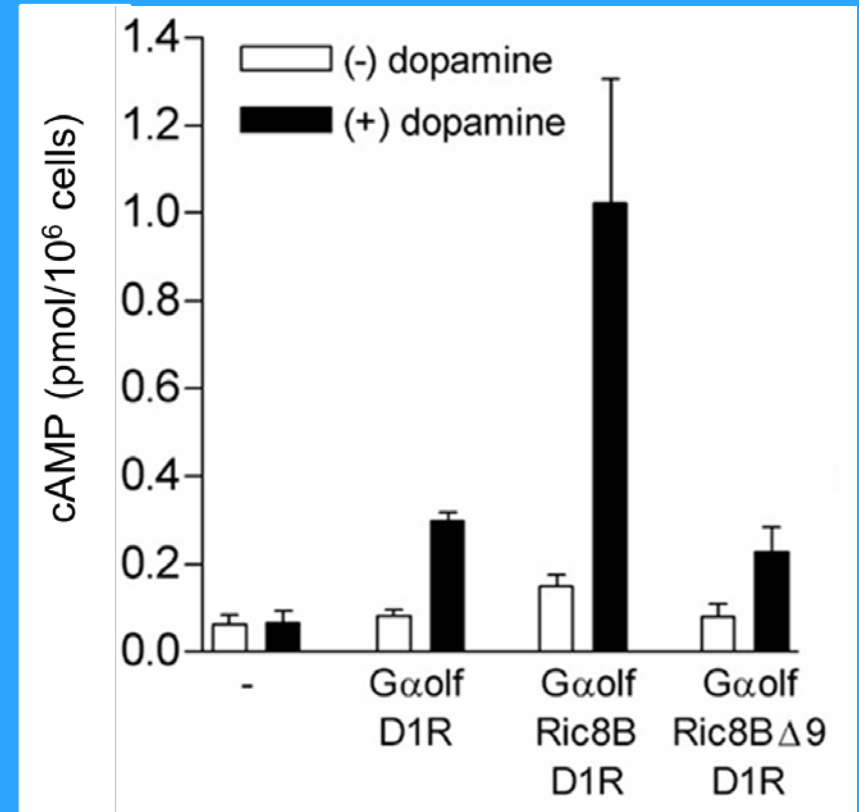
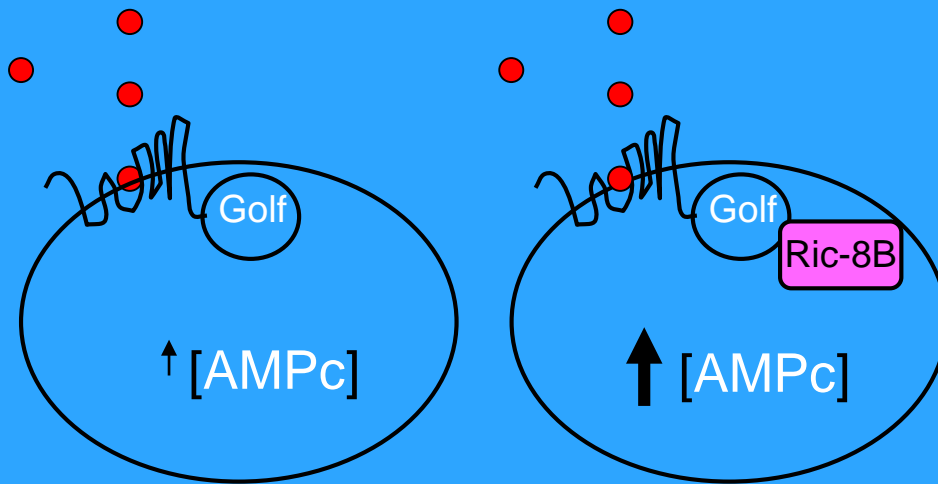


# Ric-8B is a GEF (GTP Exchange factor)

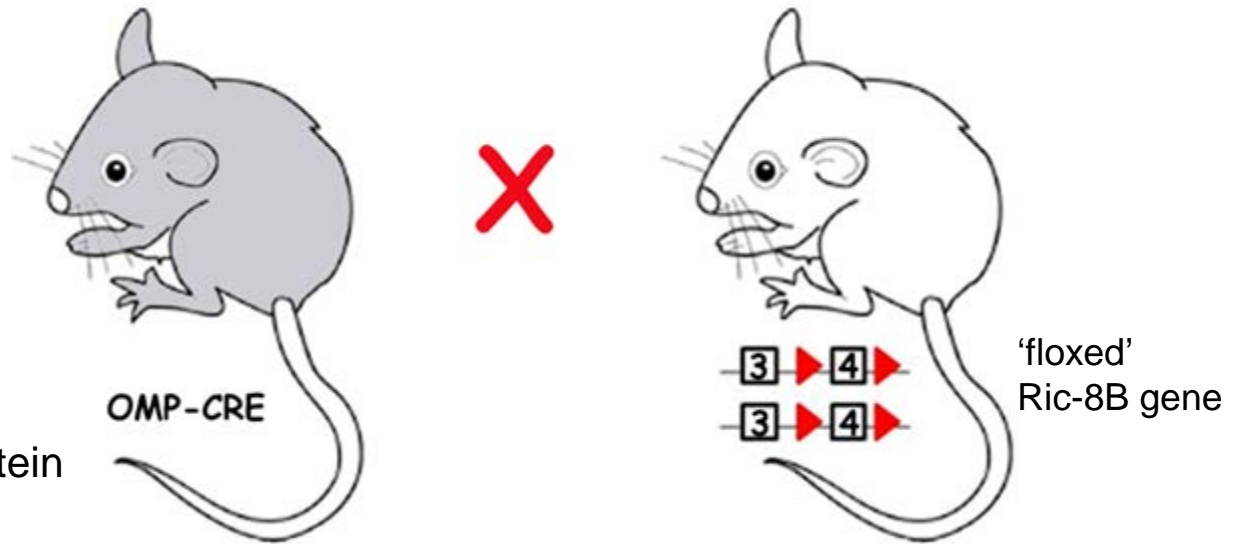




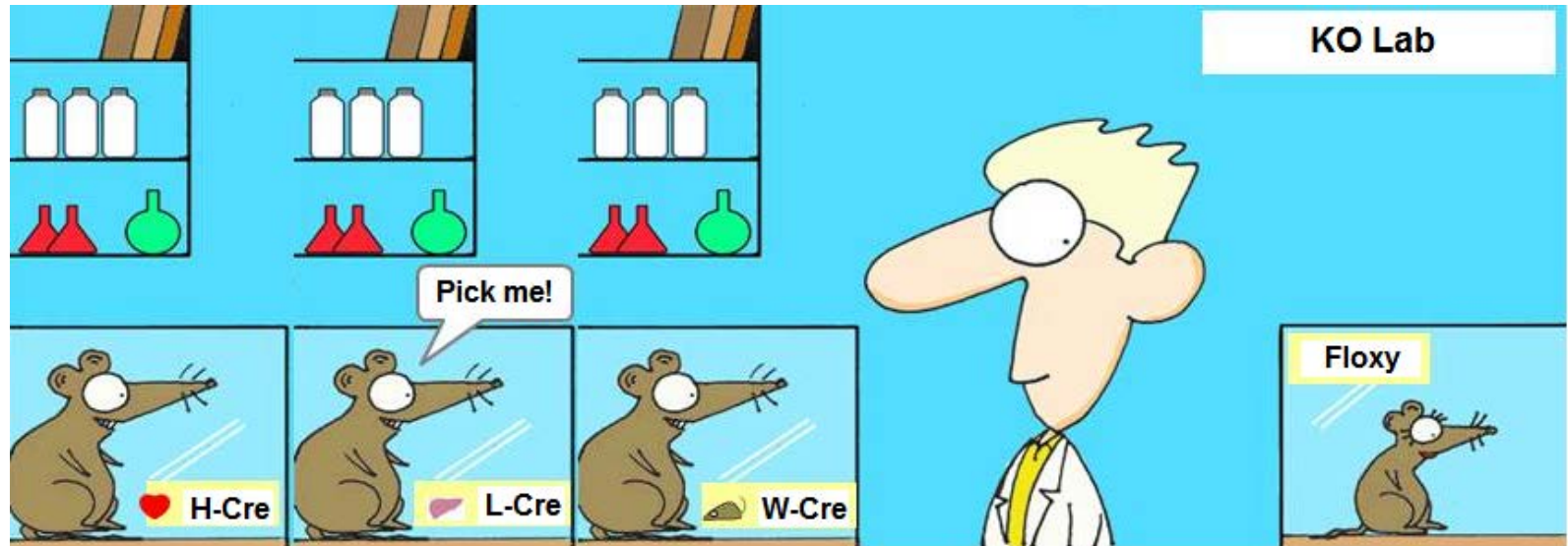
# Ric-8B amplifies signaling through $G_{\alpha olf}$ in HEK293 cells



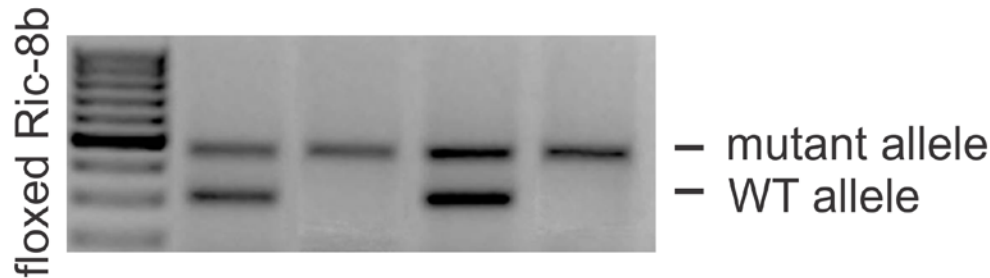
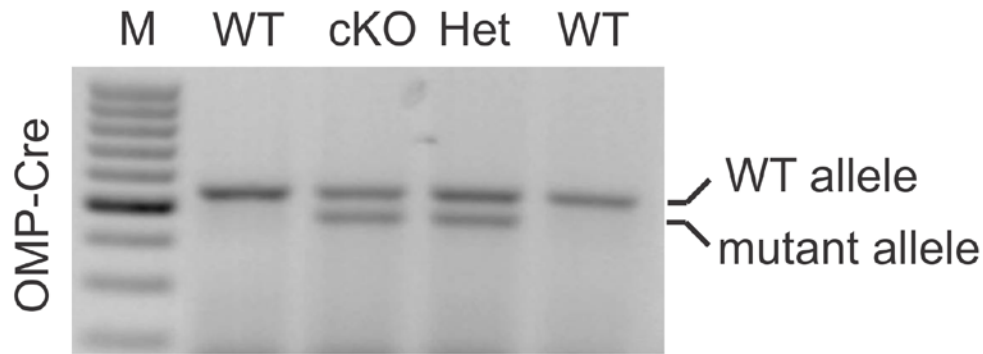
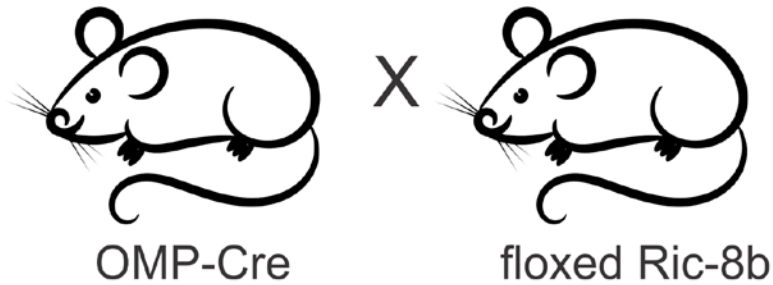
# Generation of a tissue specific Ric-8B KO using Cre-Lox



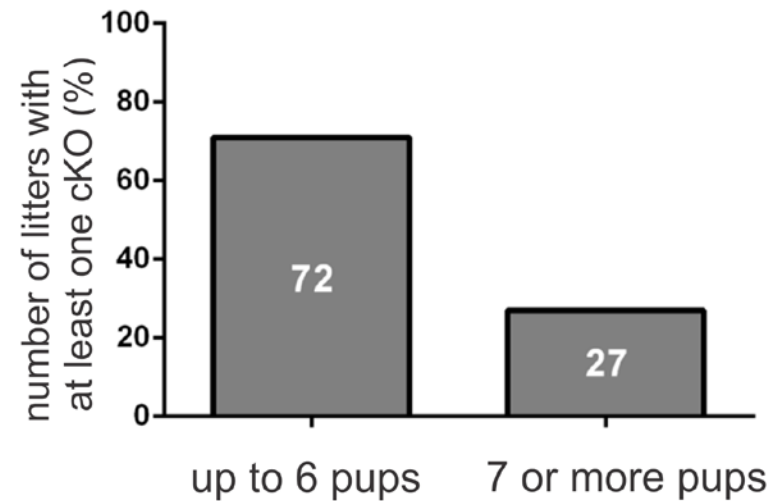
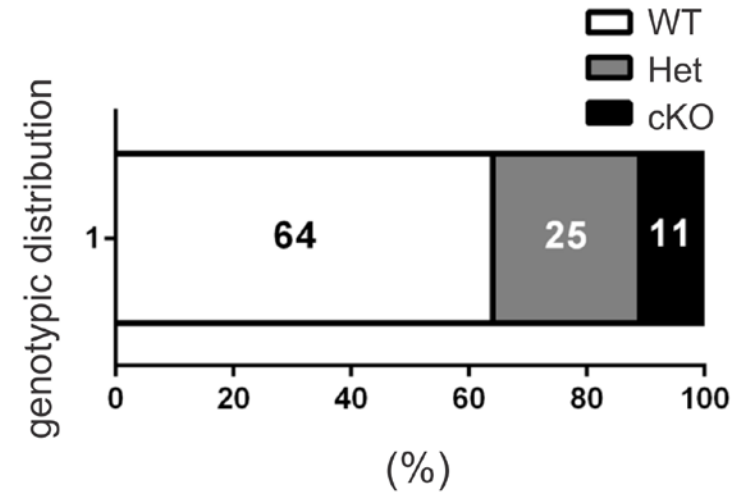
**OMP = Olfactory Marker Protein**



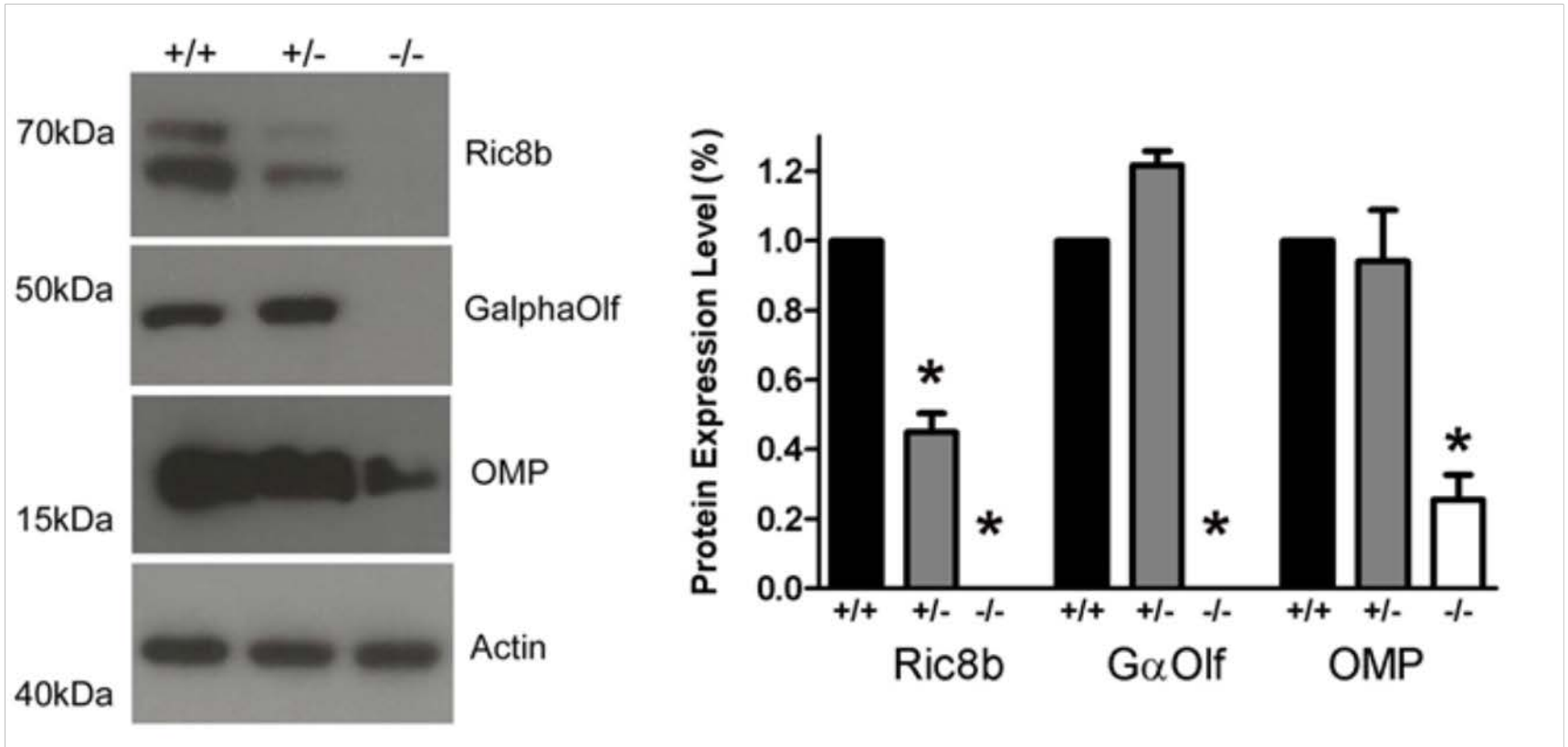
# Generation of a tissue specific Ric-8B KO using Cre-Lox



WT = Ric-8b<sup>flox/wt</sup>/OMP<sup>wt/wt</sup> or Ric-8b<sup>flox/flox</sup>/OMP<sup>wt/wt</sup>  
 Het = Ric-8b<sup>flox/wt</sup>/OMP<sup>Cre/wt</sup>  
 cKO = Ric-8b<sup>flox/flox</sup>/OMP<sup>Cre/wt</sup>

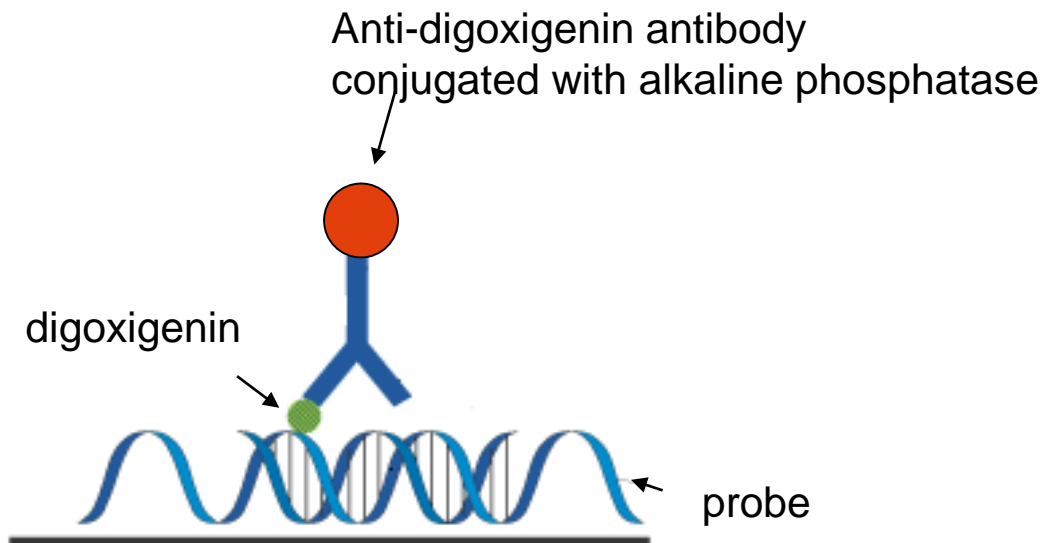


# Ric-8B KO olfactory epithelium does not express G $\alpha$ olf protein

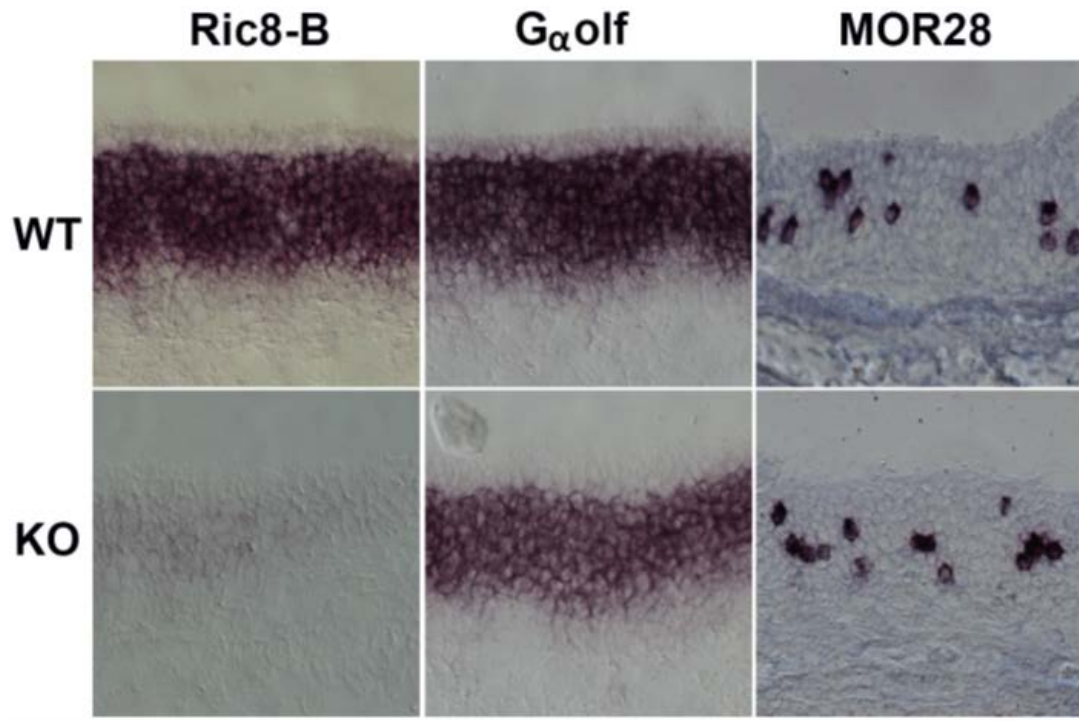


Western blot

# *In situ* hybridization



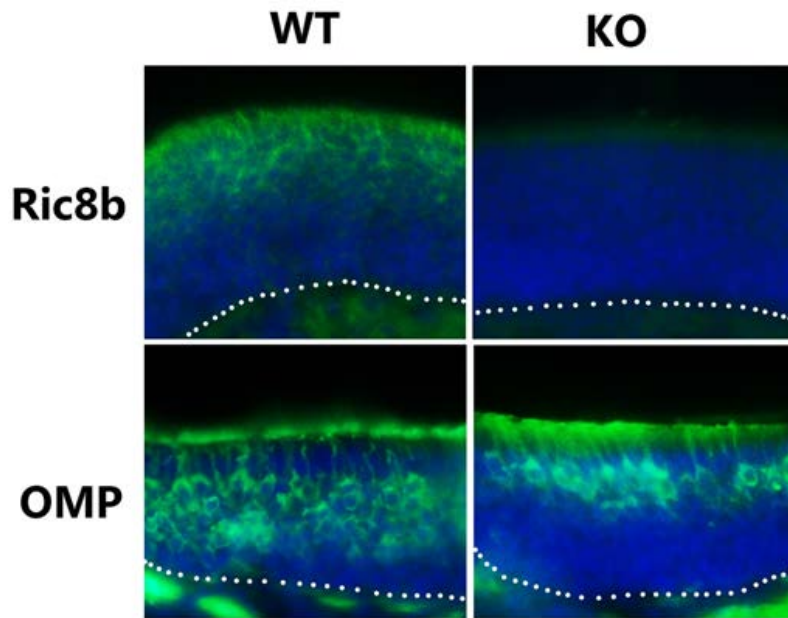
The  $G_{\alpha}olf$  gene is transcribed in Ric-8B KO mice



*In situ* hybridization



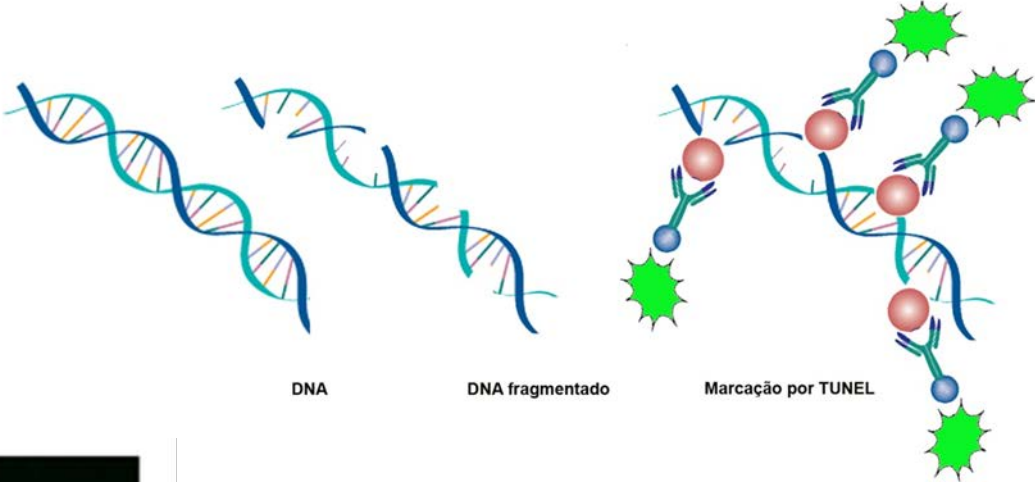
The number of olfactory neurons is reduced in Ric-8B KO mice



Immunofluorescence

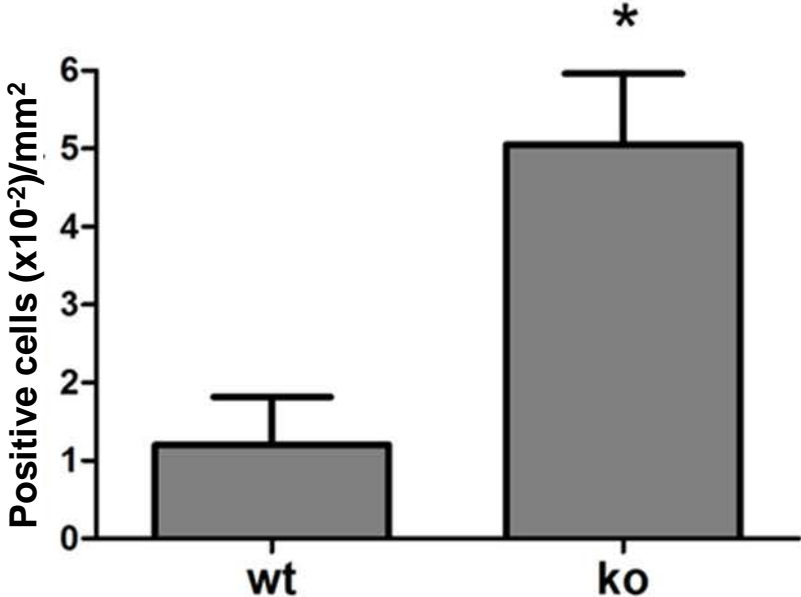
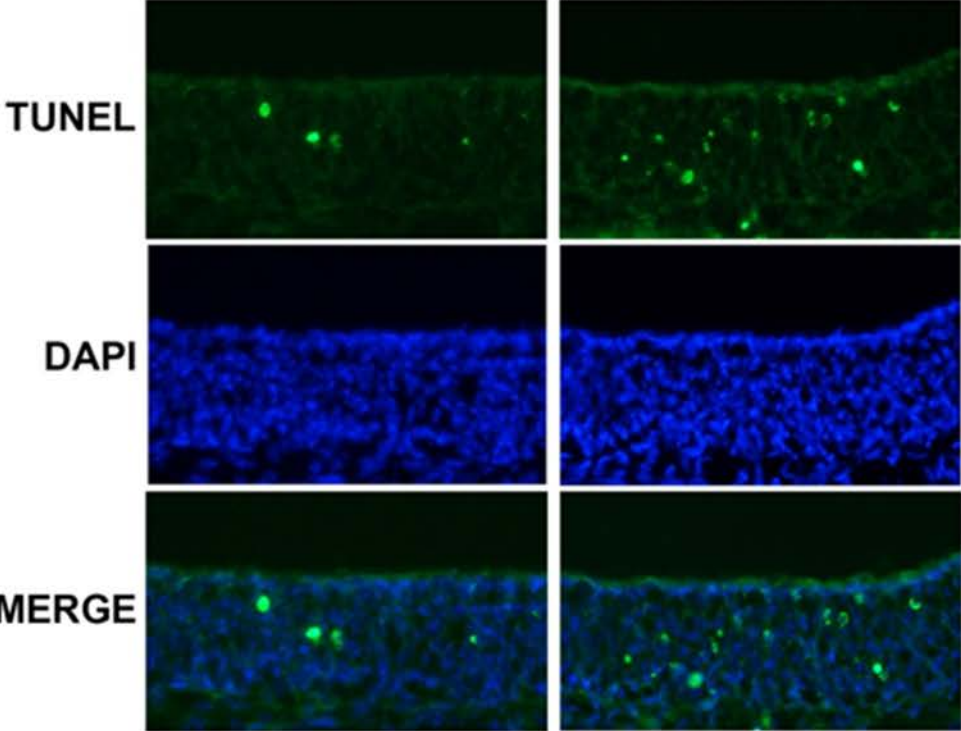


# KO mice show increased cell death of olfactory neurons

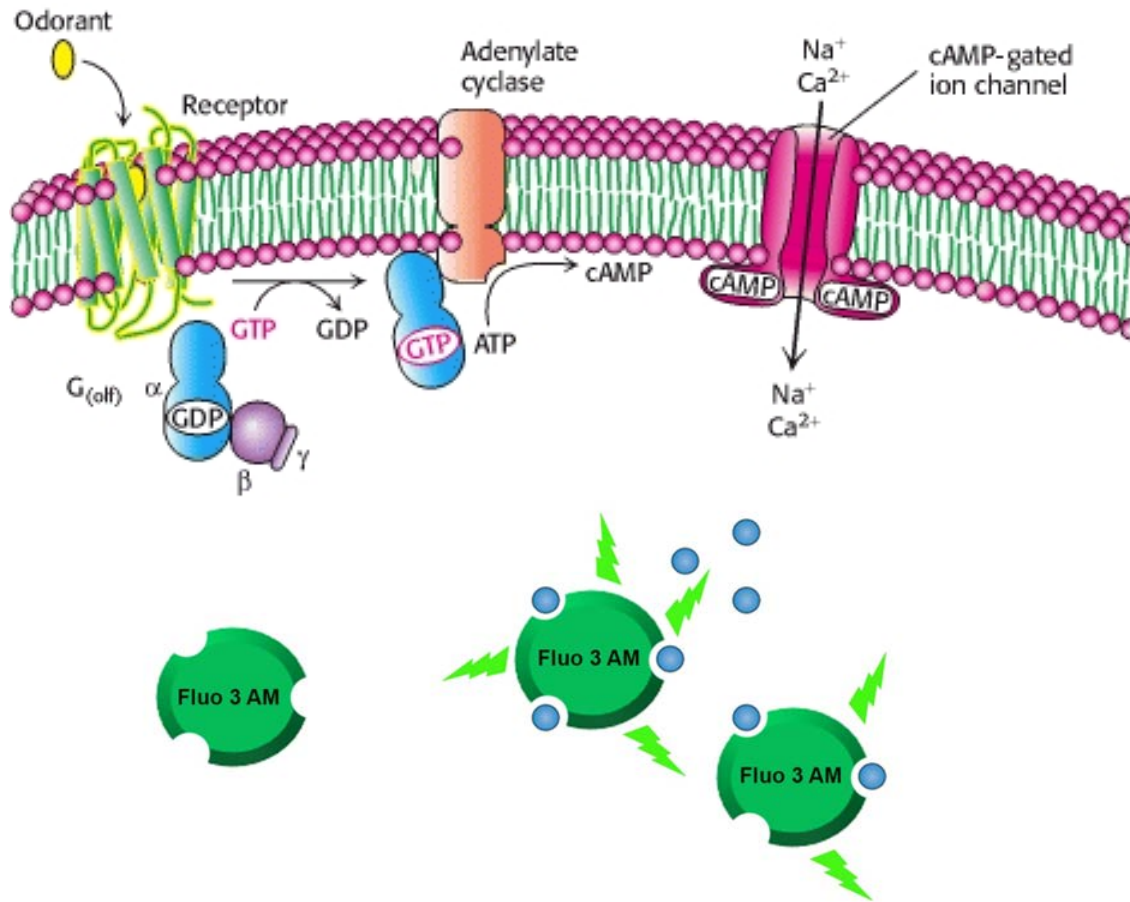


WT

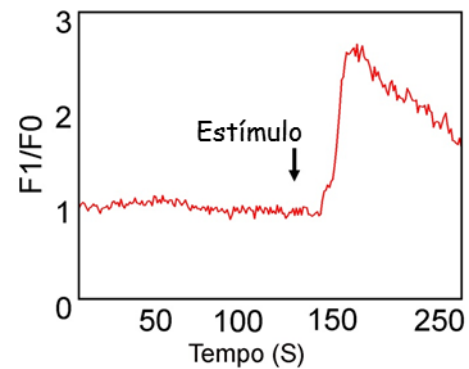
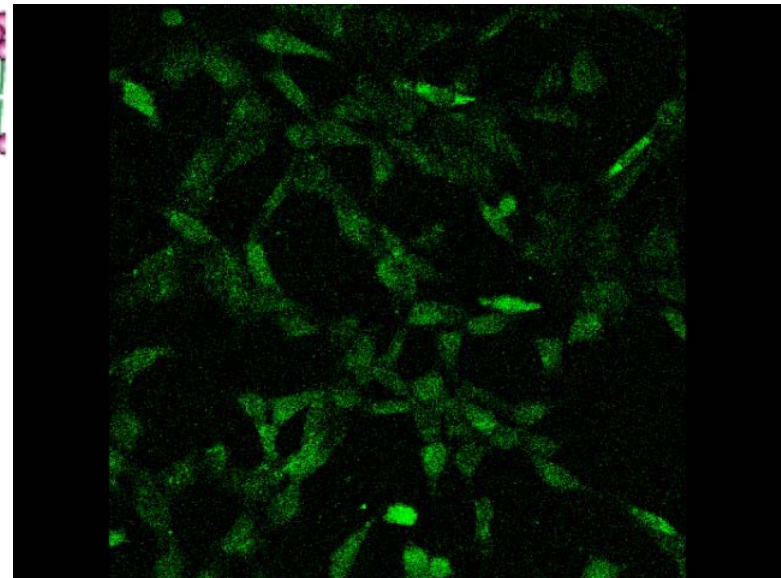
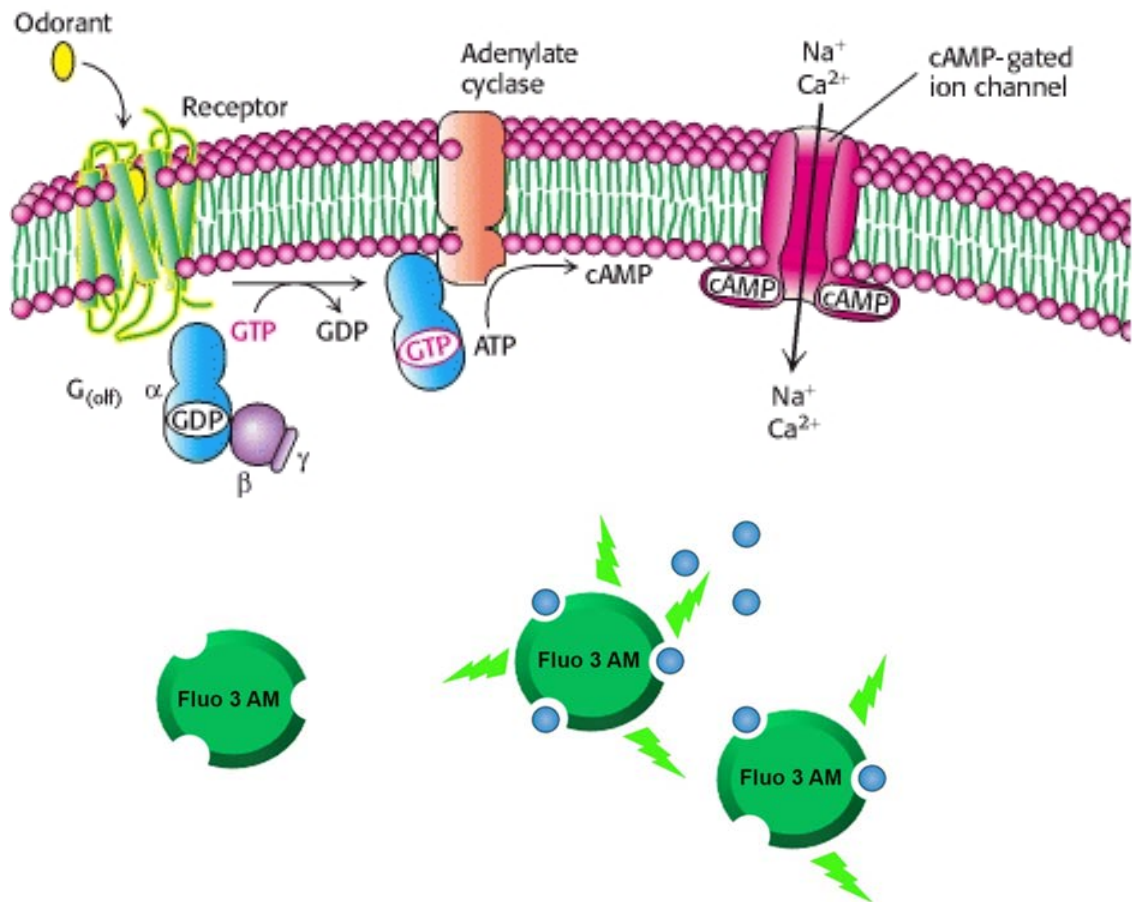
KO



# Calcium imaging

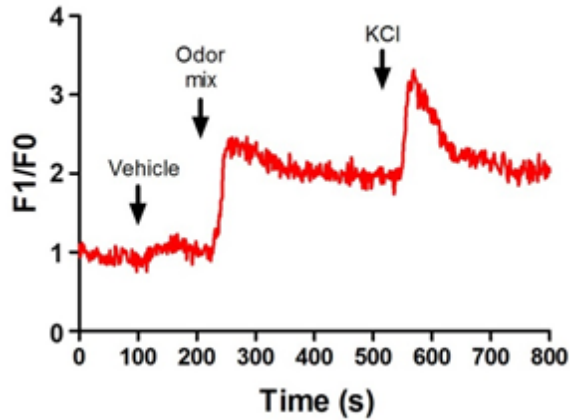


# Calcium imaging

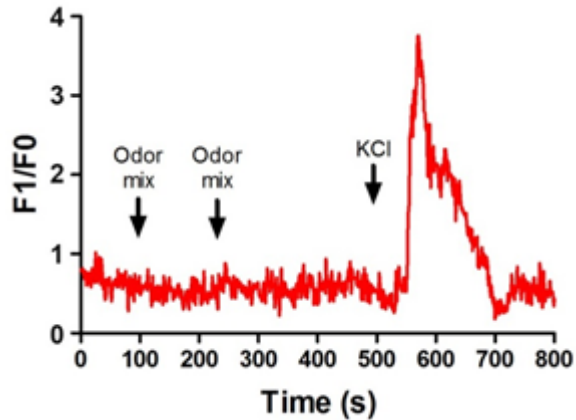


# Olfactory neurons from cKO mice are not responsive to odorants

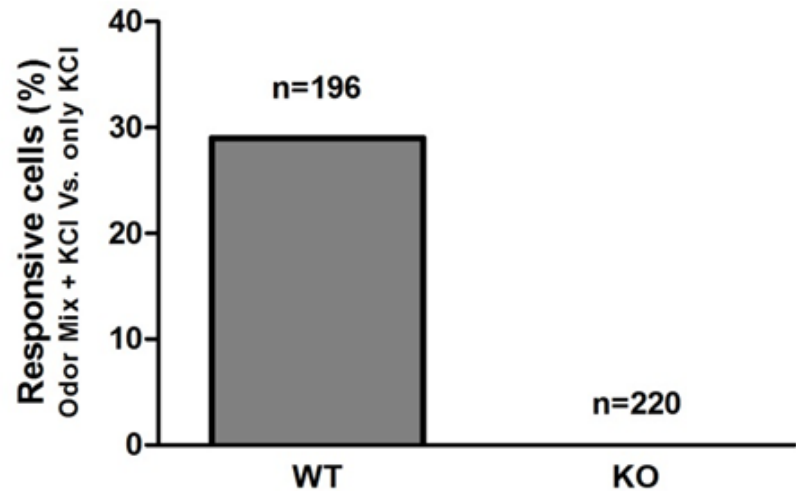
WT



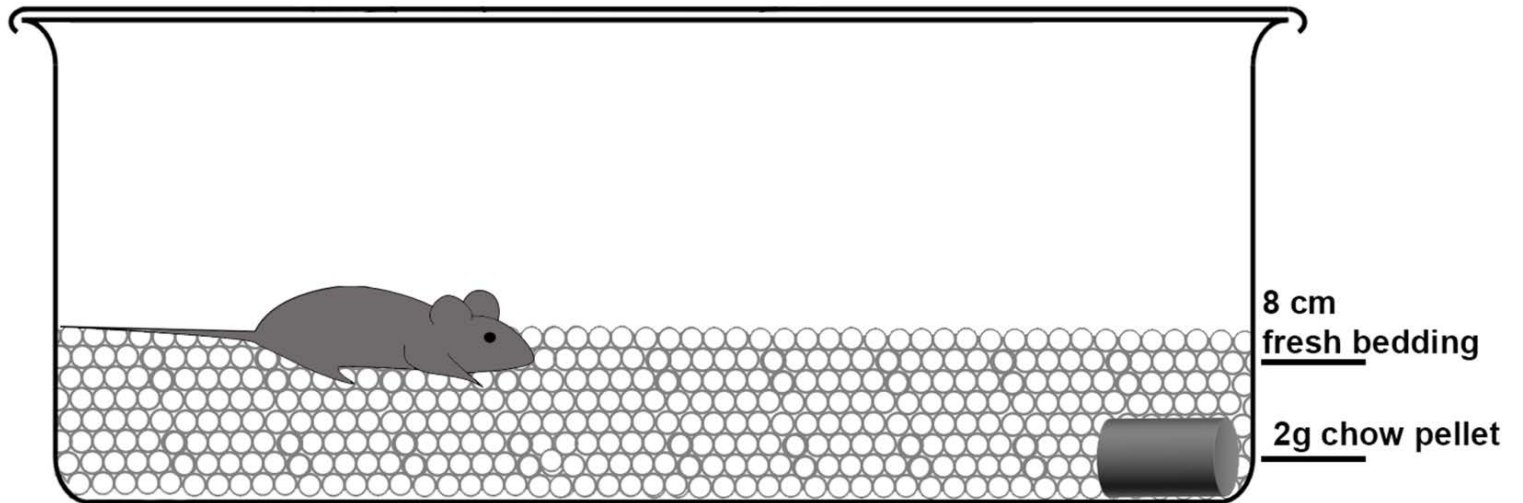
cKO



Odorantes (Mix)
Hexanal
Heptanal
Eugenol
1-Heptanol
(±) Citronellal
1-Nonanol
S(-) Limonene
Acetophenone
(±) Carvone

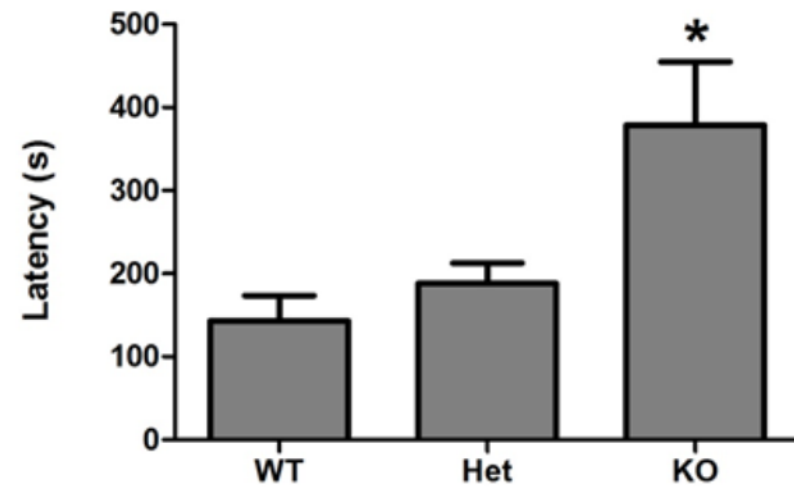


# Buried food test





# Ric8-B cKO mice show impaired olfactory behavior



Buried food test

# SUMMARY:

- Ric-8B is required for  $G\alpha_{olf}$  expression in olfactory neurons;
- Ric-8B (and  $G\alpha_{olf}$ ) are required for survival of olfactory neurons;
- Ric-8B knockout mice are anosmic.

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**Thiago Reis**

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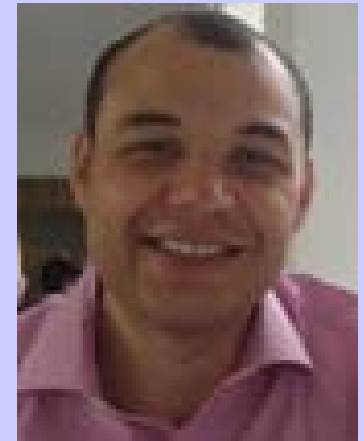
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